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Genetic Variants of the Non-homologous End Joining Gene *LIG4* and Severe Radiation Pneumonitis in Non-small Cell Lung Cancer Patients Treated with Definitive Radiotherapy

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Abstract

Background—Non-homologous end joining (NHEJ) is a pathway that repairs DNA double-strand breaks (DSBs) to maintain genomic stability in response to irradiation. We hypothesized that single nucleotide polymorphisms (SNPs) in NHEJ repair genes may affect clinical outcomes among non-small cell lung cancer (NSCLC) patients treated with definitive radio(chemo)therapy.

Methods—We genotyped five potentially functional SNPs (i.e., *XRCC4* rs6869366 [-1394G>T] and rs28360071 [intron 3, del/ins], *XRCC5* rs3835 [2408G>A], *XRCC6* rs2267437 [-1310C>G] and *LIG4* rs1805388 [T9I]) and estimated their associations with severe radiation pneumonitis (RP, ≥ grade 3) in 195 NSCLC patients.

Results—We found a predictive role of *LIG4* rs1805388 SNP in RP development (adjusted hazard ratio [HR] = 2.08, 95% confidence interval [CI], 1.04–4.12, *P* = 0.037 for CT/TT vs. CC). In addition, male patients with the TT genotype of *XRCC4* rs6869366 SNP and female patients with AG/AA genotypes of *XRCC5* rs3835 SNP were also at increased risk of severe RP development.

Conclusions—Our results suggest that NHEJ genetic polymorphisms, particularly *LIG4* rs1805388, may modulate the risk of radiation pneumonitis in NSCLC patients treated with definitive radio(chemo)therapy. Large studies are needed to confirm our findings.

Keywords

Radiation pneumonitis; Polymorphism; Non-small cell lung cancer

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Conflict of Interest Disclosures: None.

Introduction

Radiation therapy is one of the major treatment modalities for unresectable non-small cell lung cancer (NSCLC) and has been shown to improve local control and overall survival. However, acute radiation treatment-related pulmonary toxicity, known as radiation pneumonitis (RP), is the most common dose-limiting complication of thoracic radiation, with 10% to 20% of patients experiencing severe RP.¹ Some therapeutic and patient-related factors are found to be associated with RP risk, including patient performance status, dosimetric parameters, and plasma inflammatory cytokine levels.² In addition, genetically determined cellular responses to ionizing radiation have also been suggested, because patients receiving similar treatment could have a different response to radiotherapy. Recently, we demonstrated that a single nucleotide polymorphism (SNP) of the *TGF β 1* gene (rs1982073) independently predicted RP risk in patients with NSCLC.³ Considering the relatively sparse reports of RP investigation from a genetic perspective, we were interested in identifying additional genetic factors that are likely to predict RP susceptibility.

Ionizing radiation can produce a wide variety of lesions in cellular DNA, among which the DNA double-strand breaks (DSBs) are the principle genotoxic lesions that pose major threats to genomic integrity. It is estimated that a dose of ~1 Gy of X-rays produces about 50-100 double-strand breaks in the DNA of a typical mammalian cell, leading to 50% cell death.^{4, 5} There are two main pathways for DNA DSB repair—homologous recombination and non-homologous end-joining (NHEJ). In NHEJ, a relatively small number of essential repair proteins mediate the DSB repair, including XRCC4, XRCC5 (Ku80), XRCC6 (Ku70), DNA Ligase4 and NEHJ1 (XLF). The initial step involves recognition and signaling of DSB by the ring-shaped heterodimer Ku70/Ku80 (known as the Ku complex); then the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) interacts with Ku in a DNA-dependent manner to form a fully functional DNA-PK holoenzyme that functions in the synapsis of two broken DNA ends; finally, XRCC4 and DNA ligase4 form a tight protein complex that catalyzes the rejoining of two compatible DNA ends; another core NHEJ protein, XLF, might function by stimulating the basic NHEJ ligation reaction, but its precise function is still unknown.⁶

NHEJ is the major pathway to repair DSBs in mammalian species. Previous studies have demonstrated that loss of Ku or XRCC4/Ligase4 function compromises the rejoining of radiation-induced DSBs and leads to increased radiosensitivity.^{7, 8} Patients of a rare congenital disorder, the Ligase IV syndrome, are extremely sensitive to radiation due to mutations in the DNA ligase4 gene.⁹ Hence, it is reasonable to speculate that the inter-individual variability in NHEJ may modulate phenotype of radiosensitivity, hence affecting clinical outcomes of radiotherapy.

Since SNPs can contribute directly to disease predisposition by modifying a gene's function, or they can serve as genetic markers for nearby disease-causing variants through association or linkage disequilibrium (LD), we hypothesized that functional SNPs of the NHEJ genes are biomarkers for predicting susceptibility to RP among patients with lung cancer treated with thoracic radiation. To test this hypothesis, we performed a case-only study, seeking associations between RP risk and common functional variants of *XRCC4*, *XRCC5*, *XRCC6* and *LIG4* in NSCLC patients who received definitive radiation therapy, with or without chemotherapy.

Methods and Materials

Patient Population

We identified 261 patients with DNA samples available from a data set of 576 patients with NSCLC treated with definitive radiation at The University of Texas M. D. Anderson Cancer Center between 1999 and 2005. Among these 261 patients, 195 patients had documented information on RP with complete follow-up information and radiation dosimetric data. The median total radiation dose was 63 Gy (range, 50.4 to 84.0 Gy) given at 1.2 to 2.0 Gy/fraction. Of all patients, 20 patients received radiation only, 175 received radiation in combination with chemotherapy. Details of the radiation treatment planning, follow-up schedule and tests, guidelines for RP scoring, and dosimetric data analysis have been described elsewhere.^{10, 11}

Briefly, all patients were examined by their treating radiation oncologists weekly during concurrent chemoradiotherapy and 4–6 weeks after completion of treatment. The patients were then followed every 3 months for the first 3 years and every 6 months thereafter unless they had symptoms that required immediate examination or intervention. Radiographic examination by chest X-ray or computerized tomography was performed at each follow-up visit after completion of chemoradiotherapy. For this analysis, we reviewed all relevant dictated clinical notes by the treating physicians and all radiographic images for every patient. Radiation treatment-related pneumonitis was diagnosed by clinical presentation and any of the following radiographic abnormalities: ground-glass opacity, attenuation, or consolidation changes within the radiation field. Pneumonitis was assessed and scored using the Common Terminology Criteria for Adverse Events version 3.0.¹² The time to the end point development was calculated from the beginning of the radiation therapy; patients not experiencing the end point were censored at the last follow-up. This study was approved by the appropriate M. D. Anderson Cancer Center institutional review board.

Genotyping Methods

We selected five common, well-studied and potentially functional variants of *XRCC4*, *XRCC5*, *XRCC6* and *LIG4* genes involved in NHEJ: *XRCC4* (rs6869366 [-1394G>T]) and rs28360071 [intron 3, del/ins]), *XRCC5* (rs3835 [2408G>A]), *XRCC6* (rs2267437 [-1310C>G]) and *LIG4* (rs1805388 [T9I]). These SNPs were selected, because they met at least two of three criteria: (1) a minor allele frequency of at least 5% in Caucasians, (2) to be located in the promoter region or splicing site or to cause amino acid changes, (3) previously reported to be associated with cancer risk or survival.^{13,14,15-17} Although the *XLF* gene is also important in the NHEJ response to DSB,⁶ no functional SNPs have been reported so far, and therefore this gene was not included in the present study. Genomic DNA was extracted from the buffy coat fraction of each blood sample by using a Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA purity and concentrations were determined by spectrophotometric measurement of absorbance at 260 and 280 nm by a UV spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE). Genotypes were generated by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, and two research assistants independently read the gel pictures, and the genotyping was repeated, if there was a disagreement of the result. We also selected 10% of the samples for replication, and the results were 100% concordant. The primer sequences, restriction enzymes and PCR conditions used for the experiments are shown in Table 1.

Statistical Analysis

Patients were grouped according to their genotypes. Statistical analysis was performed using the SAS 9.2 statistical software (SAS Inc, Chicago, IL). Cox proportional hazards analysis

was performed to evaluate the effect of genotypes on RP risk by calculating the hazard ratios (HRs) and 95% confidence intervals (CIs). Kaplan-Meier analysis was performed to estimate the cumulative RP probability. In addition, multivariate Cox regression analysis was performed to adjust for other covariates. Likelihood ratio tests were performed for each of multivariate Cox regression models to assess the goodness of fit. A two-sided test with a *P* value of 0.05 or less was considered statistically significant.

Finally, we used a classification and regression tree (CART) analysis to identify higher-order interactions between clinical factors and genetic variants using the rpart package in S-PLUS Version 8.0.4 (TIBCO, Palo Alto, CA). CART creates a decision tree to identify how well each genotype or environmental factor variable predicts the class (e.g. RP). Specifically, the recursive procedure starts at the root node and uses a log-rank statistic to determine the first optimal split and each subsequent split of the data set. This process continues until the terminal nodes have no subsequent statistically significant splits or the terminal nodes reach a pre-specified minimum size ($n > 10$).

Results

Patient Characteristics and Association with Radiation Pneumonitis

Table 2 lists the characteristics of the 195 patients (112 men and 83 women with a median age of 63 years [range, 35 to 88 years]), of whom 71.3% were white, 85.1% had stage III/IV disease, 89.7% were treated with a combination of chemotherapy and radiation therapy, and 96.8% received radiation doses between 60 and 70 Gy. To determine if any confounding factors were influencing the risk of RP, we analyzed the associations between RP and clinicopathologic characteristics including age, sex, race, Karnofsky Performance Score (KPS), disease stage, tumor histology, smoking history, use of chemotherapy, radiation dose and mean lung dose (MLD). The median follow-up time for RP of any grade after radiation therapy, with or without chemotherapy, was 21 months and the median time for RP (grade ≥ 3) development was 3.5 months (95% CI, 3.3-4.5 months). We found that sex and MLD were significantly associated with severe RP (grade ≥ 3) in the univariate analysis (HR for male vs. female 2.28, 95% CI 1.07-4.86, $P = 0.034$; and HR for MLD ≥ 19.7 vs. MLD < 19.7 2.16, 95% CI 1.04-4.50, $P = 0.040$). None of the other clinicopathologic characteristics were associated with severe RP risk in this study population.

Radiation Pneumonitis and Genotype Association

Table 3 shows the results of univariate and multivariate analyses of the associations between genetic polymorphisms and grade ≥ 3 RP by the Cox proportional hazards analysis. In the univariate analysis, the G allele of *XRCC4* rs6869366 SNP showed a tendency towards a decreasing risk of severe RP (GT vs. TT 0.51, 95% CI 0.12-2.13, $P = 0.356$), whereas the *LIG4* rs1805388 T allele was associated with a significantly increased risk of severe RP (CT + TT vs. CC 1.96, 95% CI 1.00-3.85, $P = 0.048$). Similar results were found in multivariate analyses performed with adjustment of sex, KPS, TNM tumor stage, and MLD (Table 3), which were potential confounding factors of RP, as suggested by the current and previous studies.^{2,3} Fig. 1 plots the incidence of RP grade ≥ 3 as a function of time since radiation therapy according to selected clinical factors and genetic polymorphisms. We further assessed these associations in the white patients only ($n=139$) and found similar tendency of associations (data not shown).

Association of Multiple Factor Interaction with RP Risk (CART analysis)

To assess potential interactions on the risk of developing RP, we built a decision tree using clinicopathological variables (sex, disease stage, KPS group, and MLD) and all SNPs included in the current study. In the CART analysis, the first split in the decision tree was

MLD, indicating that MLD was the strongest predictor for RP among the factors considered. Further inspection of the tree structure showed distinct patterns between low MLD (< 19.7Gy) and high MLD (≥ 19.7 Gy) groups (Fig. 2). In the low MLD group, compared with those carrying the wild-type *LIG4* CC genotype, patients with CT/TT genotypes had a significantly higher risk of developing severe RP (HR = 6.34, 95% CI 1.64-24.51). In the high MLD group, sex was the second split in the decision tree. Male patients with the TT genotype of *XRCC4* rs6869366 SNP and female patients with AG/AA genotypes of *XRCC5* rs3835 SNP showed significantly higher risk of developing severe RP, compared with the reference group (HR = 8.67, 95% CI 2.55-29.47; and HR = 4.69, 95% CI 1.05-20.96, respectively) (Fig. 2).

Discussion

To the best of our knowledge, this is the first report of an association between potentially functional SNPs of the NHEJ genes and risk of developing severe RP in lung cancer patients treated with radiotherapy, with a reasonable sample size (a total of 195). Our findings suggest some statistical evidence that *LIG4* rs1805388 may independently modulate the risk of developing severe RP in patients treated with radiotherapy, and thus if validated, such genetic variants could be a useful biomarker for susceptibility to RP in NSCLC patients under consideration for radiotherapy.

Exposure of cells to ionizing radiation leads to the generation of DNA DSBs, which cause disastrous consequences if left unrepaired or misrepaired. Two main pathways are involved in the repair of DNA DSBs in eukaryotic or mammalian cells, including the homologous recombination and NHEJ. Different from homologous recombination that requires extensive homology and the *RAD52* epistasis group of genes, NHEJ does not require homology but is greatly facilitated by the DNA-PKc and the Ligase4/XRCC4 complex. Although both pathways have been shown to be active from yeast to humans, their significance varies substantially between higher and lower eukaryotes. In yeast, the majority of IR-induced DNA DSBs are removed by homologous recombination, whereas in humans, NHEJ appears to be the dominant pathway of removing IR-induced DNA DSBs.¹⁸

The classical model of the NHEJ-mediated DSB repair suggests that the progression of NHEJ reaction from one event to the next is carried out by sequential recruitment of the core proteins that play specific roles. However, the most recent investigation suggests that NHEJ proceeds in the presence of all core proteins that are pre-assembled into a functional machinery at the site of DSBs.⁶ Despite all the uncertainties, some core proteins are believed to have more directly physical interactions as detected by biochemical methods, including the XRCC5/XRCC6 (Ku) complex with a high affinity for DSB and the Ligase4/XRCC4 complex responsible for the ligation.

To date, the five NHEJ SNPs included in the current study have been found to be associated with different types of cancer susceptibility, including cancers of the lung (*XRCC4* rs6869366),¹⁴ breast (*XRCC6* rs2267437 and *XRCC5* rs3835),¹⁵ colorectum (*XRCC4* rs6869366),¹⁶ and oral cavity (*XRCC4* rs2836071).¹⁷ However, their associations with radiation treatment-related toxicities have been rarely investigated. In our current study, *LIG4* rs1805388 had a significant association with development of severe RP in patients undergoing thoracic irradiation for NSCLC, independent of patient-, tumor-, and therapy-related factors. This SNP causes a nonsynonymous amino acid change from threonine to isoleucine at the N-terminal of the *LIG4* protein, which is essential for its activity.¹⁹ Previous study reported that the substitution led to around 50% decrease in the adenylation and double-strand ligation activity of ligase IV,^{20,21} which may result in an increased radiosensitivity as observed in the current study. In addition, the *XRCC4* rs6869366 SNP,

located in the promoter region of that gene, also showed a tendency to confer the RP susceptibility, although statistical significance was not reached probably because of the reduced study power. Considering functional relevance of these two proteins, the subsequent changes in the activity of the Ligase4/XRCC4 complex resulting from a functional change caused by SNPs may pose a substantial influence on the whole NHEJ machinery. Indeed, although LIG4 primarily interacts with XRCC4 through the region located between the BRCA1 and BRCT domain,¹⁹ previous study found a significant gene-gene interaction between *LIG4* and *XRCC4* in modulation of lung cancer risk as mediated by *LIG4* rs1805388 SNP.¹³ Taken together, *LIG4* rs1805388 SNP may influence the NHEJ capacity by regulating both its own activity and interaction with the XRCC4 protein. However, the current study did not have the statistical power to evaluate such an interaction, and additional mechanistic investigations are needed, if our findings are validated in larger studies.

Our CART analysis was an explorative, non-parametric approach, which requires no assumption of a genetic model. Through this decision tree-based data mining, we confirmed MLD, sex and *LIG4* rs1805388 to be the most important risk factors for severe RP development in this study population, because they were the first and second split in the decision tree. The *XRCC4* rs6869366 TT genotype with MLD ≥ 19.7 Gy in males, *XRCC5* rs3835 AG/AA genotypes with MLD ≥ 19.7 Gy in females, and *LIG4* rs1805388 CT/TT genotypes with MLD < 19.7 Gy were most likely to be associated with severe RP. These findings suggest that those patients with such genotypes may require close surveillance after thoracic radiotherapy. Once again, these findings need to be validated in larger studies.

Despite these positive findings, our current study has some limitations. First, we were not able to explore the mechanism by which the NHEJ genetic polymorphisms lead to RP in lung cancer patients. Secondly, we used a candidate polymorphism approach, which allowed us to focus on potentially functional SNPs reported in the literatures but did not comprehensively cover all SNPs in the entire gene. Some important but rare SNPs may have been missed or the observed association may result from genetic linkages with other untyped SNPs. These warrant additional investigations of the tagging SNPs in the future, which may help find out the underlying disease-causing variants. Thirdly, we did not adjust the p-values for multiple tests, simply because this was an exploratory study with limited study power. We plan to confirm current findings in our ongoing prospective expansion studies in more stringent conditions with larger sample sizes.

In summary, the present study indicated that polymorphisms of the NHEJ gene *LIG4* had a significant effect on the risk of developing severe RP in patients who received definitive radiation therapy for NSCLC; and that *XRCC4* rs6869366 and *XRCC5* rs3835 might be involved in the interaction in the risk of developing RP. Once validated, *LIG4* SNPs could be a useful biomarker for susceptibility to RP. Therefore, larger independent studies are needed to validate these findings.

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References

1. Roach M 3rd, Gandara DR, Yuo HS, et al. Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. *J Clin Oncol*. 1995; 13(10):2606–2612. [PubMed: 7595714]
2. Provatopoulou X, Athanasiou E, Gounaris A. Predictive markers of radiation pneumonitis. *Anticancer Res*. 2008; 28(4C):2421–2432. [PubMed: 18751429]
3. Yuan X, Liao Z, Liu Z, et al. Single nucleotide polymorphism at rs1982073:T869C of the TGFbeta 1 gene is associated with the risk of radiation pneumonitis in patients with non-small-cell lung cancer treated with definitive radiotherapy. *J Clin Oncol*. 2009; 27(20):3370–3378. [PubMed: 19380441]
4. Nikjoo H, O'Neill P, Wilson WE, Goodhead DT. Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. *Radiat Res*. 2001; 156(5 Pt 2):577–583. [PubMed: 11604075]
5. Ward JF. The yield of DNA double-strand breaks produced intracellularly by ionizing radiation: a review. *Int J Radiat Biol*. 1990; 57(6):1141–1150. [PubMed: 1971840]
6. Yano K, Morotomi-Yano K, Adachi N, Akiyama H. Molecular mechanism of protein assembly on DNA double-strand breaks in the non-homologous end-joining pathway. *J Radiat Res (Tokyo)*. 2009; 50(2):97–108. [PubMed: 19346677]
7. Ayene IS, Ford LP, Koch CJ. Ku protein targeting by Ku70 small interfering RNA enhances human cancer cell response to topoisomerase II inhibitor and gamma radiation. *Mol Cancer Ther*. 2005; 4(4):529–536. [PubMed: 15827325]
8. Bertolini LR, Bertolini M, Anderson GB, Maga EA, Madden KR, Murray JD. Transient depletion of Ku70 and Xrcc4 by RNAi as a means to manipulate the non-homologous end-joining pathway. *J Biotechnol*. 2007; 128(2):246–257. [PubMed: 17097754]
9. Chistiakov DA, Voronova NV, Chistiakov AP. Ligase IV syndrome. *Eur J Med Genet*. 2009; 52(6):373–378. [PubMed: 19467349]
10. Wang S, Liao Z, Wei X, et al. Analysis of clinical and dosimetric factors associated with treatment-related pneumonitis (TRP) in patients with non-small-cell lung cancer (NSCLC) treated with concurrent chemotherapy and three-dimensional conformal radiotherapy (3D-CRT). *Int J Radiat Oncol Biol Phys*. 2006; 66(5):1399–1407. [PubMed: 16997503]
11. Jin H, Tucker SL, Liu HH, et al. Dose-volume thresholds and smoking status for the risk of treatment-related pneumonitis in inoperable non-small cell lung cancer treated with definitive radiotherapy. *Radiother Oncol*. 2009; 91(3):427–432. [PubMed: 18937989]
12. CTEP. Common terminology criteria for adverse events, version 3. 2003.
13. Tseng RC, Hsieh FJ, Shih CM, Hsu HS, Chen CY, Wang YC. Lung cancer susceptibility and prognosis associated with polymorphisms in the nonhomologous end-joining pathway genes: a multiple genotype-phenotype study. *Cancer*. 2009; 115(13):2939–2948. [PubMed: 19408343]
14. Hsu NY, Wang HC, Wang CH, et al. Lung cancer susceptibility and genetic polymorphism of DNA repair gene XRCC4 in Taiwan. *Cancer Biomark*. 2009; 5(4):159–165. [PubMed: 19729825]
15. Willems P, Claes K, Baeyens A, et al. Polymorphisms in nonhomologous end-joining genes associated with breast cancer risk and chromosomal radiosensitivity. *Genes Chromosomes Cancer*. 2008; 47(2):137–148. [PubMed: 18000863]
16. Bau DT, Yang MD, Tsou YA, et al. Colorectal cancer and genetic polymorphism of DNA double-strand break repair gene XRCC4 in Taiwan. *Anticancer Res*. 30(7):2727–2730. [PubMed: 20683005]
17. Chiu CF, Tsai MH, Tseng HC, et al. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. *Oral Oncol*. 2008; 44(9):898–902. [PubMed: 18164646]
18. Jeggo PA. DNA breakage and repair. *Adv Genet*. 1998; 38:185–218. [PubMed: 9677708]
19. Grawunder U, Zimmer D, Leiber MR. DNA ligase IV binds to XRCC4 via a motif located between rather than within its BRCT domains. *Curr Biol*. 1998; 8(15):873–876. [PubMed: 9705934]

20. Girard PM, Kysela B, Harer CJ, Doherty AJ, Jeggo PA. Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. *Hum Mol Genet.* 2004; 13(20):2369–2376. [PubMed: 15333585]
21. O'Driscoll M, Cersaletti KM, Girard PM, et al. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell.* 2001; 8(6):1175–1185. [PubMed: 11779494]

Figure 1.

Risk of radiation pneumonitis (grade ≥ 3) by selected clinical factors and polymorphisms of DNA double-strand break genes. (A) and (B), radiation pneumonitis by sex and mean lung dose; (C), radiation pneumonitis by *LIG4* rs1805388 SNP. The P values were obtained from the Cox hazards model without adjustment.

Figure 2. Classification and regression tree analysis for predictors of grade ≥ 3 radiation pneumonitis in 195 patients with NSCLC. SNPs were classified as wild type (W) and variant genotype (V). *LIG4*: rs1805388; *XRCC5*: rs3835; *XRCC4*: rs6869366.

Table 1
PCR-RFLP Conditions for Selected SNPs

Gene	SNP	Primer sequence	Temp	Enzyme
<i>XRCC4</i>	rs6869366	Forward: 5'-GATGCGAACTCAAAGATACTGA-3' Reverse: 5'-TGTAAGCCAGTACTCAAACCTT-3'	55°C	HincII
	rs28360071	Forward: 5'-TCCTGTTACCATTTTCAGTGTAT-3' Reverse: 5'-CACCTGTGTTCAATTCAGCTT-3'	58°C	None
<i>XRCC5</i>	rs3835	Forward: 5'-CCAGTCATTGAGTTCTCTGC-3' Reverse: 5'-AACACTAGAGCTGCATCCTG-3'	61°C	AluI
	rs2267437	Forward: 5'-CTTCAGACCACTCTCTTCTC-3' Reverse: 5'-TCACCTCACAGTAGTCGTTG-3'	58°C	HhaI
<i>LIG4</i>	rs1805388	Forward: 5'-TCTGTATTCGTTCTAAAGTTGAACA-3' Reverse: 5'-TGCTTTACTAGTTAAACGAGAAGAT-3'	52°C	HpyCH4III

Table 2
Patient Demographics and Association with RP (grade $\geq$ 3) (N=195)

Parameter	No. (%)	HR	95% CI	$P^{\dagger}$
Sex				
Female	83 (42.6)	1.00		
Male	112 (57.4)	2.28	1.07-4.86	0.034
Age (years)				
<63	96 (49.2)	1.00		
$\geq$ 63	99 (50.8)	1.23	0.63-2.40	0.565
Race				
White	139 (71.3)	1.00		
Black	41 (21.4)	0.68	0.26-1.77	0.430
Other	15 (7.7)	1.45	0.44-4.83	0.531
KPS				
<80	40 (20.5)	1.00		
$\geq$ 80	155 (79.5)	0.61	0.27-1.41	0.248
Stage groups				
III, IV	166 (85.1)	1.00		
I, II	29 (14.9)	0.97	0.40-2.34	0.942
Histology				
Adenocar	66 (33.7)	1.00		
NSCLC, NOS	67 (34.2)	1.17	0.54-2.53	0.692
Squamous cell	62 (32.1)	0.77	0.33-1.84	0.561
Smoking status				
Ever	176 (90.7)	1.00		
Never	18 (9.3)	1.36	0.77-2.40	0.292
Chemotherapy				
No	20 (10.3)	1.00		
Yes	175 (89.7)	1.35	0.41-4.43	0.617
Mean lung dose (Gy)				
<19.7	88 (45.1)	1.00		
$\geq$ 19.7	107 (54.9)	2.16	1.04-4.50	0.040
Radiation dose (Gy)				
<63	36 (18.5)	1.00		
$\geq$ 63	159 (81.5)	0.71	0.31-1.63	0.423

† P values were calculated by Cox proportional model using univariate analysis.

Table 3
Univariate and Multivariate Analyses of Different Genotypes and RP (grade≥3) for NSCLC

Genotypes	Patient No.	Event	Crude		P*	Adjusted		P†
			HR	95% CI		HR	95% CI	
XRCC4 (rs6869366 -1394G>T)								
TT	175	34	1.00			1.00		
GT	19	2	0.51	0.12-2.13	0.356	0.43	0.10-1.80	0.247
GG	0	0	N/A					
GG+GT	19	2	0.51	0.12-2.13	0.356	0.43	0.10-1.80	0.247
XRCC4 (rs28360071 del/ins)								
ins/ins	82	16	1.00			1.00		
ins/del	110	20	0.87	0.45-1.69	0.672	0.82	0.42-1.62	0.572
del/del	3	0	N/A					
ins/del+del/del	113	20	0.84	0.43-1.63	0.606	0.81	0.41-1.59	0.534
XRCC5 (rs3835 2408G>A)								
GG	102	17	1.00			1.00		
AG	80	17	1.26	0.64-2.48	0.501	1.50	0.75-2.98	0.250
AA	11	1	0.55	0.07-4.13	0.562	0.56	0.07-4.25	0.573
AG+AA	91	18	1.20	0.61-2.36	0.593	1.39	0.70-2.77	0.346
XRCC6 (rs2267437 -1310C>G)								
CC	85	17	1.00			1.00		
CG	75	12	0.92	0.43-1.95	0.827	0.93	0.44-1.99	0.878
GG	35	7	0.98	0.40-2.39	0.970	0.79	0.32-1.96	0.607
CG+GG	110	19	0.93	0.48-1.81	0.824	0.86	0.44-1.67	0.647
LIG4 (rs1805388 T9I)								
CC	142	21	1.00			1.00		
CT	43	12	1.96	0.96-3.98	0.064	1.98	0.96-4.07	0.063
TT	9	3	1.96	0.46-8.38	0.356	3.64	0.82-16.11	0.088

Genotypes	Patient No.	Event	Crude			Adjusted		
			HR	95% CI	P*	HR	95% CI	P†
CT+TT	52	15	1.96	1.00-3.85	0.048	2.08	1.04-4.12	0.037

* P values were calculated by Cox proportional model using univariate analysis.

† P values were calculated with adjustment for sex, KPS score, tumor stage, and mean lung dose.